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<p>(54) Title: CYSTEINE RICH RECEPTORS: TRAIL</p> <p>(57) Abstract</p> <p>Novel receptors in the TNF family: TRAIL-R2 and TRAIL-R3.</p> <p style="text-align: center;">Abs 8, 9, 12 against Abs 14-15</p>			

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CYSTEINE RICH RECEPTORS: TRAIL

Background of the Invention

The present invention relates to novel receptors in the TNF family. Two novel receptors have been identified, TRAIL-R3 and TRAIL-R2.

5 The TNF family consists of pairs of ligands and their specific receptors referred to as TNF family ligands and TNF family receptors (Bazzoni and Beutler, 1996). The family is involved in the regulation of the immune system and possibly other non-immunological systems. The regulation is often at a "master switch" level such that TNF family signaling can result in a large number of subsequent events best
10 typified by TNF. TNF can initiate the general protective inflammatory response of an organism to foreign invasion that involves the altered display of adhesion molecules involved in cell trafficking, chemokine production to drive specific cells into specific compartments and the priming of various effector cells. As such, the regulation of these pathways has clinical potential.

15 The TNF receptor family is a collection of related proteins that generally consist of an extracellular domain, a transmembrane domain and an intracellular signaling domain. The extracellular domain is built from 2-6 copies of a tightly disulphide bonded domain and is recognized on the basis of the unique arrangement of cysteine residues. Each receptor binds to a corresponding ligand although one ligand may share
20 several receptors. In some cases, it is clear that by alternate RNA splicing, soluble forms of the receptors lacking the transmembrane region and intracellular domain exist naturally. Moreover, in nature, truncated versions of these receptors exist and the soluble inhibitory form may have direct biological regulatory roles. Clearly, viruses have used this tactic to inhibit TNF activity in their host organisms (Smith, 1994).
25 These receptors can signal a number of events including cell differentiation, cell death or cell survival signals. Cell death signaling often is triggered via relatively direct links to the caspase cascade of proteases e.g. Fas and TNF receptors. Most receptors in this class can also activate NFKB controlled events.

30 The receptors are powerful tools to elucidate biological pathways via their easy conversion to immunoglobulin fusion proteins. These dimeric soluble receptor forms are good inhibitors of events mediated by either secreted or surface bound ligands. By binding to these ligands they prevent the ligand from interacting with cell associated receptors that can signal. Not only are these receptor-Ig fusion proteins useful in an

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experimental sense, but they have been successfully used clinically in the case of TNF-R-Ig to treat inflammatory bowel disease, rheumatoid arthritis and the acute clinical syndrome accompanying OKT3 administration (Eason et al., 1996; Feldmann et al., 1996; van Dullemen et al., 1995). One can envision that manipulation of the
5 many events mediated by signaling through the TNF family of receptors will have wide application in the treatment of immune based diseases and also the wide range of human diseases that have pathological sequelae due to immune system involvement. A soluble form of a recently described receptor, osteoprotegerin, can block the loss of bone mass and, therefore, the events controlled by TNF family receptor signaling are
10 not necessarily limited to immune system regulation. Antibodies to the receptor can block ligand binding and hence can also have clinical application. Such antibodies are often very long-lived and may have advantages over soluble receptor-Ig fusion proteins which have shorter blood half-lives.

While inhibition of the receptor mediated pathway represents the most exploited
15 therapeutic application of these receptors, originally it was the activation of the TNF receptors that showed clinical promise (Aggarwal and Natarajan, 1996). Activation of the TNF receptors can initiate cell death in the target cell and hence the application to tumors was and still is attractive (Eggermont et al., 1996). The receptor can be activated either by administration of the ligand, i.e. the natural pathway or some
20 antibodies that can crosslink the receptor are also potent agonists. Antibodies would have an advantage in oncology since they can persist in the blood for long periods whereas the ligands generally have short lifespans in the blood. As many of these receptors may be expressed more selectively in tumors or they may only signal cell death or differentiation in tumors, agonist antibodies could be good weapons in the
25 treatment of cancer. Likewise, many positive immunological events are mediated via the TNF family receptors, e.g. host inflammatory reactions, antibody production etc. and therefore agonistic antibodies could have beneficial effects in other, non-oncological applications.

Paradoxically, the inhibition of a pathway may have clinical benefit in the
30 treatment of tumors. For example the Fas ligand is expressed by some tumors and this expression can lead to the death of Fas positive lymphocytes thus facilitating the ability of the tumor to evade the immune system. In this case, inhibition of the Fas system could then allow the immune system to react to the tumor in other ways now that

access is possible (Green and Ware, 1997).

The receptors are also useful to discover the corresponding ligand as they can serve as probes of the ligand in expression cloning techniques (Smith et al., 1993).

- 5 Likewise, the receptors and ligands can form in vitro binding assays that will allow the identification of inhibitory substances. Such substances can form the basis of novel inhibitors of the pathways.

Brief Description of the Drawings

- 10 Figure 1 provides the sequence of TRAIL R-2.
Figure 2 provides the sequence of TRAIL R-3.
Figure 3 is a Northern blot showing expression of TRAIL-R2.
Figure 4 is a Northern blot showing expression of TRAIL-R3.
Figure 5 shows the sequence alignment of TRAIL receptors 1-3.

15 **Detailed Description**

A. DEFINITIONS

- "Homologous", as used herein, refers to the sequence similarity between sequences of molecules being compared. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, e.g., if a
20 position in each of two DNA molecules is occupied by adenine, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared x 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences
25 are 60% homologous. By way of example, the DNA sequences ATTGCC and TATGCC share 50% homology. Generally, a comparison is made when two sequences are aligned to give maximum homology.

- A "purified preparation" or a "substantially pure preparation" of a polypeptide, as used herein, means a polypeptide that has been separated from other proteins, lipids,
30 and nucleic acids with which it naturally occurs. Preferably, the polypeptide is also separated from other substances, e.g., antibodies, matrices, etc., which are used to purify it.

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"Transformed host" as used herein is meant to encompass any host with stably integrated sequence, i.e. TRAIL R2 or TRAIL R3 sequence, introduced into its genome or a host possessing sequence, i.e. receptor encoding episomal elements.

A "treatment", as used herein, includes any therapeutic treatment, e.g., the
5 administration of a therapeutic agent or substance, e.g., a drug.

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A "substantially pure nucleic acid", e.g., a substantially pure DNA, is a nucleic acid which is one or both of: (1) not immediately contiguous with either one or both of the sequences, e.g., coding sequences, with which it is immediately contiguous (i.e., one at the 5' end and one at the 3' end) in the naturally-occurring genome of the organism from which the nucleic acid is derived; or (2) which is substantially free of a nucleic acid sequence with which it occurs in the organism from which the nucleic acid is derived. The term includes, for example, a recombinant DNA which is incorporated into a vector, e.g., into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other DNA sequences. Substantially pure DNA also includes a recombinant DNA which is part of a hybrid gene encoding one of the claimed TRAIL-R sequences.

The terms "peptides", "proteins", and "polypeptides" are used interchangeably herein.

"Biologically active" as used herein, means having an in vivo or in vitro activity which may be performed directly or indirectly. Biologically active fragments of TRAIL receptors may have, for example, 70% amino acid homology with the active site of the receptors, more preferably at least 80%, and most preferably, at least 90% homology. Identity or homology with respect to the receptors is defined herein as the percentage of amino acid residues in the candidate sequence which are identical to the TRAIL-R residues in SEQ. ID. NO. 2 or 4..

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature.

The present invention is related to two novel receptors for TRAIL have been identified, designated TRAIL-R2 and TRAIL-R3 (collectively referred to herein as TRAIL receptors). Their ligand, TRAIL, shows a broad tissue distribution.

As used herein, the terms "TRAIL receptor" and "TRAIL-R" refer to proteins having amino acid sequences which are substantially similar to the native mammalian TRAIL-R2, and -R3 receptor amino acid sequences, set forth in SEQ. ID. NOS. 2 and 4, and which are biologically active, as defined below, in that they are capable of

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binding to ligands or transducing a biological signal initiated by a TRAIL ligand binding to a cell, or cross-reacting with anti-TRAIL-R antibodies raised against TRAIL-R. The terms as used herein include, but are not limited to, analogs or subunits of native proteins having at least 70-90% homology and which exhibit at least some biological activity in common with TRAIL-R2 and TRAIL-R3, for example, soluble constructs which are devoid of a transmembrane region but retain the ability to bind to a ligand. Various bioequivalent protein and amino acid analogs are described in detail below.

The TRAIL receptors of the invention may be isolated from mammalian tissues and purified to homogeneity, or isolated from cells which contain membrane-bound TRAIL-R, and purified to homogeneity. Methods for growing cells and isolating cell extracts are well known in the art, as are various cell types and growth and isolation methods. In general, any TRAIL-R can be isolated from any cell or tissue expressing this protein using a cDNA probe, isolating mRNA and transcribing the mRNA into cDNA. Thereafter, the protein can be produced by inserting the cDNA into an expression vector, such as a virus, plasmid, cosmid or other expression vector, inserting the expression vector into a cell, and proliferating the resulting cells. The TRAIL-R can then be isolated from the medium or cell extract by methods well known in the art. One skilled in the art can readily vary the vectors and cell lines and still obtain the claimed receptors.

Alternatively, TRAIL-receptors may be chemically synthesized using the sequences set forth in SEQ. ID. NO. 2 and SEQ. ID. NO. 4.

TRAIL-R2 is structurally similar to the death-domain containing receptor TRAIL-R1 and is capable of inducing . The cytoplasmic domain of TRAIL-R2 binds to the adaptor molecules FADD and TRADD, and can also associate with TRAIL-R1, suggesting that TRAIL may signal through a TRAIL-R1/TRAIL-R2 heteroreceptor signalling complex. TRAIL-R3 is a putative glycosylphosphatidylinositol-anchored protein, which is either cell-associated or processed and secreted. Secreted TRAIL-R3 competes for the binding of TRAIL to TRAIL-R1 and/or TRAIL-R2, thereby acting as an inhibitor of apoptosis. TRAIL-R2 shows a broad tissue distribution, whereas the expression of TRAIL-R3 is restricted to peripheral blood lymphocytes (PBLs) and skeletal muscle. Thus it is likely that TRAIL-R3 acts as an important regulator of TRAIL-R2 and -3 induced cell death in vivo.

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The sequences of TRAIL-R2 and TRAIL-R3 contain one and five repeat units of 15 amino acids (TAPE repeats) respectively, which are located in the extracellular domain, close to the membrane interaction site. These repeat units are responsible for the anomalous migration of the receptors on SDS-PAGE gels (the TAPE repeats migrate approximately 4 times slower than predicted) a phenomenon which has already been observed in other polypeptides containing repetitive domains.

The sequence of TRAIL-R3 also predicts several sites for post-translational modifications including signal peptide cleavage, N- and O-glycosylation and GPI addition. The predicted signal peptide cleavage site of TRAIL-R2 and TRAIL-R3 are found at corresponding positions, preceding the extracellular 2 cysteine-rich domains by approximately 25 amino acids.

TRAIL-R3 is highly glycosylated. The presence of several N-linked oligosaccharides as well as of O-linked glycans was found. Additionally, it is likely that TRAIL-R3 is anchored in the plasma membrane via a GPI structure, as predicted from the cDNA sequence and the amphiphilic nature of the mature protein.

When expressed in 293T cells, TRAIL-R3 was partly recovered as a secreted, soluble protein. This processing may be through the action of a protease or a GPI-phospholipase D. The secreted form of TRAIL-R3 probably occurs as an oligomer, given its high apparent molecular weight (700-900kD) as determined by gel permeation chromatography.

sTRAIL-R3 binds to TRAIL, and can compete for TRAIL-binding to TRAIL-1 and/or R2, thereby blocking apoptotic cell death, or other activities related to the pathway. It is likely that s TRAIL-R3 contributes to the protection of T cells from spontaneous cell death during their activation, since these cells express both TRAIL and death inducing TRAIL-receptors.

TRAIL-R3 could also block TRAIL R1 and -R2 signalling pathways by interfering with TRAIL mediated trimerization of death signalling TRAIL receptors. In fact, death receptors lacking part or all of the cytoplasmic region, including the death domain, can inhibit signal transmission of intact receptors via a dominant-negative effect, convincingly demonstrated in children suffering from human autoimmune lymphoproliferative syndrome. Membrane- anchored TRAIL-R3 can act as a dominant negative inhibitor, and thus cells expressing TRAIL-R3 would be predicted to exhibit a decreased sensitivity to TRAIL mediated cytotoxicity. However, the GPI linked

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TRAIL-R3 might also transmit distinct signals independently of the presence of TRAIL-R1 and -R2, despite the lack of a cytoplasmic domain.

Both death inducing TRAIL-receptors show a broad tissue distribution. The synthesis of TRAIL-R3 which blocks TRAIL-induced apoptosis upon its secretion may
5 therefore be important for the inhibition of TRAIL mediated cell damage.

TRAIL-R2 contains two cysteine-rich repeat units in its extracellular region, followed by a predicted hydrophobic transmembrane segment and a 209 amino acid long cytoplasmic tail containing a typical "death domain". TRAIL-R3 contains a
10 characteristic motif of 15 amino acids rich in Thr, Ala, Pro and Glu residues, before the predicted transmembrane domain, which is repeated five times within the protein (TAPE repeats). The five TAPE repeats present in TRAIL-R3 are remarkably conserved diverging in only 1 out of the 15 amino acid positions. A single TAPE repeat is also present in TRAIL-R2 at a similar location.

The present invention also encompasses DNA sequences which encode the
15 TRAIL R2 and R3 receptors. These DNA sequences are set forth in SEQ. ID. NO. 1 and 3, respectively. In other embodiments, the invention relates to sequences that have at least 50% homology with DNA sequences encoding the C terminal receptor binding domain of the ligands and hybridize to the claimed DNA sequences or fragments thereof, and which encode the TRAIL receptors having the sequences identified in
20 SEQ. ID. NO. 2 or SEQ. ID. NO. 4.

The invention in certain embodiments furthermore relates to DNA sequences encoding the TRAIL receptors where the sequences are operatively linked to an expression control sequence. Any suitable expression control sequences are useful in the claimed invention, and can easily be selected by one skilled in the art.

25 The invention also contemplates recombinant DNAs comprising a sequence encoding TRAIL receptors or fragments thereof, as well as hosts with stably integrated TRAIL-R sequences introduced into their genome, or possessing episomal elements. Any suitable host may be used in the invention, and can easily be selected by one skilled in the art without undue experimentation.

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The invention also contemplates recombinant DNAs comprising a sequence encoding TRAIL receptors or fragments thereof, as well as hosts with stably integrated TRAIL-R sequences introduced into their genome, or possessing episomal elements. Any suitable host may be used in the invention, and can easily be selected by one skilled in the art without undue experimentation.

The claimed invention in certain embodiments encompasses recombinant TRAIL-R3 and -R2. One skilled in the art can readily isolate such recombinant receptors thereby providing substantially pure recombinant TRAIL-R polypeptides. Isolated receptors of the invention are substantially free of other contaminating materials of natural or endogenous origin, and contain less than about 10- 15 % by mass of protein contaminants residual of production processes.

Mammalian Receptors within the scope of the invention also include, but are not limited to, primate, human, murine, canine, feline, bovine, ovine, equine and porcine TRAIL-R2. Mammalian Receptors can also be obtained by cross species hybridization using a single stranded cDNA derived from the human TRAIL-R2 or -R3. DNA sequences of the invention can be used as a hybridization probe to isolate Receptor cDNAs from other mammalian cDNA libraries.

Derivatives of the Receptors within the scope of the invention also include various structural forms of the proteins of SEQ. ID. NOs. 2, and 4 which retain biological activity. For example, a receptor protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction.

Receptor derivatives may also be used as immunogens, reagents in a receptor-based immunoassay, or as binding agents for affinity purification procedures of TRAIL

The present invention also includes TRAIL-R3 and TRAIL-R2 with or without associated native-pattern glycosylation. One skilled in the art will understand that the glycosylation pattern on the receptor may vary depending on the particular expression system used. For example, typically, expression in bacteria such as E. coli results in a non-glycosylated molecule. TRAIL-R derivatives may also be obtained by mutations of the receptors or their subunits. A mutant, as referred to herein, is a polypeptide homologous to a claimed Receptor but which has an amino acid sequence different from the native sequence due to a deletion, insertion or substitution.

Bioequivalent analogs of the Receptor proteins of the invention may be

constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, often cysteine residues can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involved modifications, for example, to enhance expression in the chosen expression system.

Soluble Receptors of the invention may comprise subunits which have been changed from a membrane bound to a soluble form. Thus, soluble peptides may be produced by truncating the polypeptide to remove, for example, the cytoplasmic tail and/or transmembrane region. Alternatively, the transmembrane domain may be inactivated by deletion, or by substitutions of the normally hydrophobic amino acid residues which comprise a transmembrane domain with hydrophilic ones. In either case, a substantially hydrophilic hydropathy profile is created which will reduce lipid affinity and improve aqueous solubility. Deletion of the transmembrane domain is preferred over substitution with hydrophilic amino acid residues because it avoids introducing potentially immunogenic epitopes. Soluble Receptors of the invention may include any number of well-known leader sequences at the N-terminus. Such a sequence would allow the peptides to be expressed and targeted to the secretion pathway in a eukaryotic system.

The invention herein provides agents, such as agonists and antagonists, directed against the claimed receptors. In certain embodiments of this invention, the agent comprises a blocking agent that comprises an antibody directed against the TRAIL-R2 or -R3 that inhibits TRAIL receptor signaling. Preferably the antibody is a monoclonal antibody. Similarly, the claimed invention encompasses antibodies and other agents which act as agonists in the TRAIL pathways.

Inhibitory anti TRAIL-R antibodies and other receptor blocking agents can be identified using screening methods that detect the ability of one or more agents either to bind to the or TRAIL-R, or ligands thereto, or to inhibit the effects of TRAIL-R signaling on cells.

One skilled in the art will have knowledge of a number of assays that measure the strength of ligand-receptor binding and can be used to perform competition assays with putative TRAIL receptor blocking agents. The strength of the binding between a receptor and ligand can be measured using an enzyme-linked immunoadsorption assay

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(ELISA) or a radioimmunoassay (RIA). Specific binding may also be measured by fluorescently labeling antibody-antigen complexes and performing fluorescence activated cell sorting analysis (FACS), or by performing other such immunodetection methods, all of which are techniques well-known in the art.

5 With any of these or other techniques for measuring receptor-ligand interactions, one skilled in the art can evaluate the ability of a blocking agent, alone or in combination with other agents, to inhibit binding of ligands to the receptor molecules. Such assays may also be used to test blocking agents or derivatives of such agents, i.e. fusions, chimeras, mutants or chemically altered forms, to optimize the
10 ability of the agent to block receptor activation.

 The receptor blocking agents of the invention in one embodiment comprise soluble TRAIL receptor molecules. Using the sequence information herein and recombinant DNA techniques well known in the art, functional fragments encoding the TRAIL receptor ligand binding domain can be cloned into a vector and expressed in an
15 appropriate host to produce a soluble receptor molecule. Soluble TRAIL receptor molecules that can compete with native TRAIL receptors for ligand binding according to the assays described herein can be selected as TRAIL receptor blocking agents.

 A soluble TRAIL receptor comprising amino acid sequences selected from those shown herein may be attached to one or more heterologous protein domains
20 ("fusion domains") to increase the *in vivo* stability of the receptor fusion protein, or to modulate its biological activity or localization.

 Preferably, stable plasma proteins -- which typically have a half-life greater than 20 hours in the circulation of a mammal-- are used to construct the receptor fusion proteins. Such plasma proteins include but are not limited to: immunoglobulins, serum
25 albumin, lipoproteins, apolipoproteins and transferrin. Sequences that can target the soluble receptors to a particular cell or tissue type may also be attached to the receptor ligand binding domain to create a specifically localized soluble receptor fusion protein.

 All or a functional fragment of the TRAIL receptor extracellular region comprising the TRAIL receptor ligand binding domain may be fused to an
30 immunoglobulin constant region like the Fc domain of a human IgG1 heavy chain. Soluble receptor -IgG fusions proteins are common immunological reagents and methods for their construction are well known in the art. (see, e.g. U.S. Patent No. 5, 225, 538).

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A functional TRAIL-R ligand binding domain may be fused to an immunoglobulin (Ig) Fc domain derived from an immunoglobulin class or subclass other than IgG1. The Fc domains of antibodies belonging to different Ig classes or subclasses can activate diverse secondary effector functions. Activation occurs when
5 the Fc domain is bound by a cognate Fc receptor. Secondary effector functions include the ability to activate the complement system, to cross the placenta and to bind various microbial proteins. The properties of the different classes and subclasses of immunoglobulins are described in the art.

Activation of the complement system initiates cascades of enzymatic reactions
10 that mediate inflammation. The products of the complement system have a variety of functions, including binding of bacteria, endocytosis, phagocytosis, cytotoxicity, free radical production and solubilization of immune complexes.

The complement enzyme cascade can be activated by the Fc domains of antigen-bound IgG1, IgG3 and Ig M antibodies. The Fc domain of IgG2 appears to be
15 less effective, and the Fc domains of IgG4, IgA, IgD and IgE are ineffective at activating complement. Thus one can select an Fc domain based on whether its associated secondary effector functions are desirable for the particular immune response or disease being treated with the receptor-fusion protein.

It if would be advantageous to harm or kill the TRAIL ligand bearing target
20 cell, one could, for example, select an especially active Fc domain (IgG1) to make the fusion protein. Alternatively, if it would be desirable to target the TRAIL receptor -Fc fusion to a cell without triggering the complement system, an inactive IgG4 Fc domain could be selected.

Mutations in Fc domains that reduce or eliminate binding to Fc receptors and
25 complement activation have been described in the art. These or other mutations can be used, alone or in combination to optimize the activity of the Fc domain used to construct the TRAIL receptor-Fc fusion protein.

One skilled in the art will appreciate that different amino acid residues forming the junction point of the receptor-Ig fusion protein may alter the structure, stability and
30 ultimate biological activity of the soluble TRAIL receptor fusion protein. One or more amino acids may be added to the C-terminus of the selected TRAIL receptor fragment to modify the junction point whit the selected fusion domain.

The N-terminus of the TRAIL receptor fusion protein may also be varied by

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changing the position at which the selected TRAIL receptor DNA fragment is cleaved at its 5' end for insertion into the recombinant expression vector. The stability and activity of each TRAIL receptor fusion protein may be tested and optimized using routine experimentation and the assays for selecting blocking agents described herein.

5 Using the TRAIL receptor binding domain sequences within the extracellular domain as shown herein, amino acid sequence variants may also be constructed to modify the affinity of the soluble TRAIL receptor molecules for their ligands. The soluble molecules of this invention can compete for binding with endogenous receptors. It is envisioned that any soluble molecule comprising a TRAIL receptor
10 ligand binding domain that can compete with native receptors for ligand binding is a receptor blocking agent that falls within the scope of the present invention.

 In other embodiments of this invention, antibodies directed against the TRAIL receptors (anti-TRAIL-R abs) function as receptor blocking agents. The antibodies of this invention can be polyclonal or monoclonal and can be modified to optimize their
15 ability to block TRAIL-R signaling, their bioavailability, stability or other desired traits.

 Polyclonal antibody sera directed against TRAIL-R are prepared using conventional techniques by injecting animals such as goats, rabbits, rats, hamsters or mice subcutaneously with TRAIL-R fusion protein in Freund's adjuvant, followed by
20 booster intraperitoneal or subcutaneous injection in incomplete Freund's. Polyclonal antisera containing the desired antibodies directed against the TRAIL receptors can then be screened by conventional immunological procedures.

 Various forms of anti-TRAIL-R abs or can also be made using standard recombinant DNA techniques. For example, "chimeric" antibodies can be constructed
25 in which the antigen binding domain from an animal antibody is linked to a human constant domain. Chimeric antibodies reduce the observed immunogenic responses elicited by animal antibodies when used in human clinical treatments.

 In addition, recombinant "humanized" antibodies which can recognize the TRAIL-R can be synthesized. Human antibodies are chimeras comprising mostly
30 human IgG sequences into which the regions responsible for specific antigen-binding have been inserted. (e.g. WO 94/04679). Animals are immunized with the desired antigen, the corresponding antibodies are isolated, and the portion of the variable region sequences responsible for specific antigen binding are removed. The animal-derived

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antigen binding regions are then cloned into the appropriate position of human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (inter species) sequences in human antibodies, and are less likely to elicit immune responses in the mammal being treated.

5 Construction of different classes of recombinant anti-TRAIL-R antibodies can also be accomplished by making chimeric or humanized antibodies comprising the anti-R variable domains and human constant domains isolated from different classes of immunoglobulins. For example, anti TRAIL-R IgM antibodies with increased antigen binding site valencies can be recombinantly produced by cloning the antigen binding
10 site into vectors carrying the human μ chain constant regions.

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling.

15 It may be desirable to increase or decrease the affinity of anti-TRAIL-R antibodies for the receptors depending on the targeted tissue type or the particular treatment schedule envisioned. For example, it may be advantageous to treat a patient with constant levels of anti-Receptor antibodies with reduced ability to signal through the pathway for semi-prophylactic treatments. Likewise, inhibitory or anti -TRAIL-R
20 antibodies with increased affinity for the receptors may be advantageous for short term treatments.

The claimed invention in yet other embodiments encompasses pharmaceutical compositions comprising an effective amount of a TRAIL-R blocking or activating agent, and pharmaceutically acceptable carriers. The compositions of the invention
25 will be administered at an effective dose to treat the particular clinical condition addressed. Determination of a preferred pharmaceutical formulation and a therapeutically efficient dose regiment for a given application is well within the skill of the art taking into consideration for example, the condition and weight of the patient, the extent of desired treatment and the tolerance of the patient for the treatment. Doses
30 of about 1 mg/kg of a soluble TRAIL-R are expected to be suitable starting points for optimizing treatment dosages.

Determination of a therapeutically effective dose can also be assessed by performing *in vitro* experiments that measure the concentration of the blocking or

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activating agent. The binding assays described herein are useful, as are other assays known in the art.

Administration of the soluble activating or blocking agents of the invention, alone or in combination, including isolated and purified forms, their salts, or
5 pharmaceutically acceptable derivative thereof may be accomplished using any of the conventionally accepted modes of administration of agents which exhibit immunosuppressive activity.

EXAMPLES:

10

Generation of Soluble Receptor Forms:

To form an receptor inhibitor for use in man, one requires the human receptor cDNA sequence of the extracellular domain. If the mouse form is known, human cDNA libraries can be easily screened using the mouse cDNA sequence and such
15 manipulations are routinely carried out in this area. With a human cDNA sequence, one can design oligonucleotide primers to PCR amplify the extracellular domain of the receptor in the absence of the transmembrane and intracellular domains. Typically, one includes most of the amino acids between the last disulfide linked "TNF domain" and the transmembrane domain. One could vary the amount of "stalk" region included to
20 optimize the potency of the resultant soluble receptor. This amplified piece would be engineered to include suitable restriction sites to allow cloning into various C-terminal Ig fusion chimera vectors. Alternatively, one could insert a stop signal at the 3' end and make a soluble form of the receptor without resorting to the use of a Ig fusion chimera approach. The resultant vectors can be expressed in most systems used in
25 biotechnology including yeast, insect cells, bacteria and mammalian cells and examples exist for all types of expression. Various human Fc domains can be attached to optimize or eliminate FcR and complement interactions as desired. Alternatively, mutated forms of these Fc domains can be used to selectively remove FcR or complement interactions or the attachment of N-linked sugars to the Fc domain which
30 has certain advantages.

Generation of Agonistic or Antagonistic Antibodies:

The above described soluble receptor forms can be used to immunize mice and

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to make monoclonal antibodies by conventional methods. The resultant mAbs that were identified by ELISA methods can be further screened for agonist activity either as soluble antibodies or immobilized on plastic in various in vitro cellular assays. Often the death of the HT29 cell line is a convenient system that is sensitive to signalling through many TNF receptors. If this line does not possess the receptor of interest, that full length receptor can be stably transfected into the HT29 line to now allow the cytotoxicity assay to work. Alternatively, such cells can be used in the Cytosensor apparatus to assess whether activation of the receptor can elicit a pH change that is indicative of a signalling event. TNF family receptors signal well in such a format and this method does not require one to know the actual biological events triggered by the receptor. The agonistic mAbs would be "humanized" for clinical use. This procedure can also be used to define antagonistic mAbs. Such mAbs would be defined by the lack of agonist activity and the ability to inhibit receptor-ligand interactions as monitored by ELISA, classical binding or BIAcore techniques. Lastly, the induction of chemokine secretion by various cells in response to an agonist antibody can form a screening assay.

Screening for Inhibitors of the Receptor-Ligand Interaction:

Using the receptor-Ig fusion protein, one can screen either combinatorial libraries for molecules that can bind the receptor directly. These molecules can then be tested in an ELISA formatted assay using the receptor-Ig fusion protein and a soluble form of the ligand for the ability to inhibit the receptor-ligand interaction. This ELISA can be used directly to screen various natural product libraries etc. for inhibitory compounds. The receptor can be transfected into a cell line such as the HT29 line to form a biological assay (in this case cytotoxicity) that can then form the screening assay.

It will be apparent to those skilled in the art that various modifications and variations can be made in the polypeptides, compositions and methods of the invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

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WE CLAIM:

1. A polypeptide defined by the amino acid sequence of SEQ. ID. NO. 2 or 4
2. A pharmaceutical composition comprising a therapeutically effective amount of
5 antibodies against TRAIL-R3 or TRAIL-R2 , and a pharmaceutically acceptable
carrier.
3. A method for preventing or reducing the severity of an immune response
comprising administering a therapeutically effective amount of a pharmaceutical
composition according to claim 2.
- 10 4. A method for treating cancer comprising administering a therapeutically effective
amount of a pharmaceutical composition according to claim 2.
5. A method for identifying a ligand to TRAIL-R2, or TRAIL-R3 comprising the steps
of (a) providing TRAIL-R2 or R3, or a fragment thereof; (b) labeling said receptor
or fragment thereof with a detectable label; (c) screening to detect ligand which
15 bind to the detectably labeled TRAIL-R2 or -R3 or fragment thereof.
6. A method for producing an antibody preparation reactive to TRAIL-R2, -R3, or
biologically active fragments thereof comprising the step of immunizing an
organism with said receptor or biologically active fragments thereof.
7. An antibody preparation reactive to TRAIL-R2, R-3 or biologically active
20 fragments thereof.
8. A pharmaceutical composition comprising an antibody preparation of claim 7, and a
pharmaceutically acceptable carrier.
9. A method of expressing TRAIL-R2 or TRAIL-R3 in a mammalian cell comprising
the steps of: (a) introducing DNA encoding TRAIL-R2 or -R3 or biologically active
25 fragments thereof into a cell; (b) allowing said cell to live under conditions such
that the gene is expressed.
10. A method of inducing cell death comprising the administration of an agent capable
of inhibiting the binding of TRAIL-R2 or R3 or biologically active fragments
thereof to their ligands.
- 30 11. A method for treating or reducing the advancement, severity or effects of an
immunological disease in a mammal comprising the step of administering a
pharmaceutical composition which comprises a therapeutically effective amount of
a TRAIL-R2, or -R3 blocking agent and a pharmaceutically acceptable carrier.

12. The method of claim 11 wherein the blocking agent is selected from the group consisting of a soluble TRAIL-R, and antibody directed against a TRAIL-R .
13. The method according to claim 12 wherein the mammal is a human.
14. A soluble TRAIL-R comprising a human immunoglobulin FC domain.

5

1 CGAGAAACCCGCAATCTTCGGCCACAAAATACACCGATGCCGATCTACTTTAA 60
 61 GGGCTGAACCCACGGGCTGAGAGACTATAGAGCGTTCCTACCGCATGGACACG 120
 N E Q R
 121 GGGACGAAACCCCGGCGCTTCGGGGCCCGGAAAGCCAGGCCAGGACCCGGA 180
 G Q B A P A A S G A R R R H G P G P R E
 181 GGGCCGGGAGCCAGGCTTGGCTCGGGTCCCGAAGACCTTGGCTGGTGGCCG 240
 A R G A R P G L R V P K T L V L V V A A
 241 GGTCTGTGTGTGTCTCAGCTGAGTCTGTCTGATCACCACCAAGACCTAGCTCCCA 300
 V L L L V S A R S A L I T Q Q D L A P Q
 301 GCACAGATGGCCCAACAAAGAGGTCAGGCCCTCAGAGGATGTCTCCACCTGG 360
 Q R V A P Q Q K R S S F S E G L C P P G
 361 ACACCATATCTCAGAAGCGGTAGAGATTGCATCTCTGCAATATGACAGGACTATAG 420
 H H I S E D G R D C I S C K Y G Q D Y S
 421 CACTCAGGAATGACCTCTTTCTGCTTGGCTGCACAGGTGTGATCAGGTGAAGT 480
 T H W N D L L F C L R C T R C D S G E V
 481 GAGCTTAAGTCCCTGACCAAGACCAAGACAGAGTGTGTGAGTGGAGAGAGGACCTT 540
 E L S P C T T T R N T V C Q C E E G T F
 541 CCGGAAGAAGATTCTCTGAGATGTCCGGAAGTGGCCACAGGTTCTCCAGAGGAT 600
 R E E D S F E M C R K C R T G C P R Q M
 601 GGTCAAGTGGTGTATGTACACCTGGAGTGACATGGAATGTGTCCAAAGATCAG 660
 V K V G D C T P W S D I E C V H E S G
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 T K H S G E A P A V E E T V T S S P Q T
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 P A S P C S L S G I I I G V T V A A V V
 781 CTGATTTGTGGCTGTGTGTGTGTGCAAGCTTTACTGTGGAGAAAGCTTCTTACCT 840
 L I V A V F V C K S L L W K K V L P Y L
 841 GAAAGGCATCTGCTCAGGTGGTGGTGGGACCTGAGCGTGTGACAGAGCTCACAAG 900
 K G I C S G G G G D P E R V D R S S Q R
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 P G E S E H L L E P A E A E R S Q R R R
 1081 GCTGCTGTTCCAGCAATGAAGGTGATCCACTGAGACTTGAGCAGTCTTCGATGA 1140
 L L V P A N E G D P T E T L R Q C F D D
 1141 CTTCGAGACTTGGTCCCTTACTCTCTGGAGCCGCTCATGAGAGCTTGGCTCTAT 1200
 F A D L V P F D S W E P L M R K L G L M
 1201 GGACAATGAGATAAGGTGGCTAAAGCTGAGGACCGGGCCACAGGACACCTTGACAC 1260
 D N E I K V A K A E A A G E R D T L Y T
 1261 GATGCTGATAAAGTGGCTCAACAAACCGGGGAGATGCTCTGTCCACACCTGTGGA 1320
 M L I K W V N K T G R D A S V H T L L D
 1321 TGGCTTGGAGACCTGGGAGAGAGACTTGGCAAGCAGAGATTGAGGACCACTGTGAG 1380
 A L E T L G E R L A K Q K I E D H L L S
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 1741 ATGCTTTATTTATTTATTTGGCTACATTTGTAAGATCATCTACAGAGTGTGTCGAC 1800
 1801 TTCACTTGAATATATATGATGAACTTTTGGCTGGGGTGGGGGAGTCTACTC 1860
 1861 TGTCTCCAGGCTGGAGTGAATGTGCAATCTTGGCTCACTATGAGCTTGCCTTCAG 1920
 1921 GCTCAAGGATCTTCCCACTCAGCCATCAAAATAGCTGGAGCCAGGCTGTCCACACC 1980
 1981 AGCCCGGCTAATTTTGTATTTGTCTGATATAGGAGTCTCTATGTGTCTCAGGCT 2040
 2041 GGTCTGGAATTCCTGACTCAAGCACTCTGCCACCTCAGACTCCCAAGCGGTGAAT 2100
 2101 AGAGGCGTGAGGCCCATGCTTGGCTTACCTTCTACTTTTATTAATCTGTATGTTAT 2160
 2161 TTTTATGAACATGAAGAACTTTTATTAATGTAATGTTTTCATGTTTGTGATGAT 2220
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 2401 GTAGTTGTATCATATGAGTGGGAGCAAGAGGATGATCACTGATGATGATGATG 2460
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 3181 TCTGCACTCCAGGCTGTGCTCTCTGCTGCTTGTGAGGAGCAGAGTGTGCTGCTCTC 3240
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 3301 CACTCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCTGCT 3360
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1 TTCGGCACGAGGCACGCGCACGAACTCAGCCAACGATTCTGATAGATTTTGGGAGTTT 60
61 GACCAGAGATGCAAGGGGTGAAGGAGCGCTTCCTACCGTTAGGAACTCTGGGACAGAGC 120
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301 AGTGGCCCCACAGCAACAGAGGCACAGCTTCAAGGGGAGGAGTGTCCAGCAGGATCTCA 360
V A P Q Q Q R H S F K G E E C P A G S H

361 TAGATCAGAACATACTGGAGCCTGTAACCCGTGCACAGAGGGTGTGGATTACACCAACGC 420
R S E H T G A C N P C T E G V D Y T N A

421 TTCCAACAATGAACCTTCTTGCTTCCCATGTACAGTTTGTAAATCAGATCAAAAACATAA 480
S N N E P S C F P C T V C K S D Q K H K

481 AAGTTCCTGCACCATGACCAGAGACACAGTGTGTCTAGTGTAAAGAAGGCACCTTCCGGAA 540
S S C T M T R D T V C Q C K E G T F R N

541 TGAAAACTCCCCAGAGATGTGCCGAACTGTAGCAGGTGCCCTAGTGGGGAAGTCCAAGT 600
E N S P E M C R K C S R C P S G E V Q V

601 CAGTAATTGTACGTCTGGGATGATATCCAGTGTGTTGAAGAATTGGTGCCAATGCCAC 660
S N C T S W D D I Q C V E E F G A N A T

661 TGTGGAAACCCAGCTGCTGAAGAGACAATGAACACCAGCCCGGGGACTCCTGCCCCAGC 720
V E T P A A E E T M N T S P G T P A P A

721 TGCTGAAGAGACAATGAACACCAGCCCAGGGACTCCTGCCCCAGCTGCTGAAGAGACAAT 780
A E E T M N T S P G T P A P A A E E T M

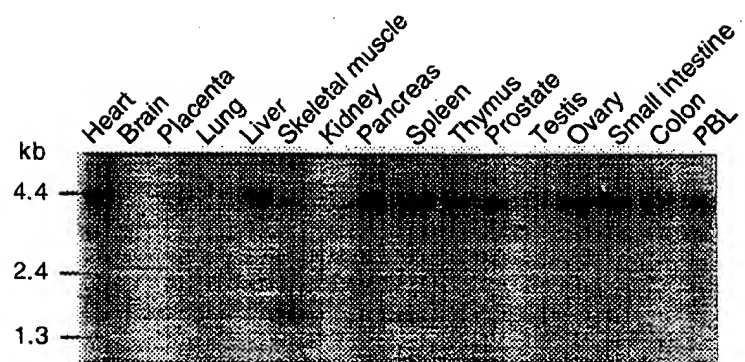
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T T S P G T P A P A A E E T M T T S P G

841 GACTCCTGCCCCAGCTGCTGAAGAGACAATGACCACCAGCCCGGGGACTCCTGCCTCTTC 900
T P A P A A E E T M T T S P G T P A S S

901 TCATTACCTCTCATGCACCATCGTAGGGATCATAGTTCTAATTGTGCTTCTGATTGTGTT 960
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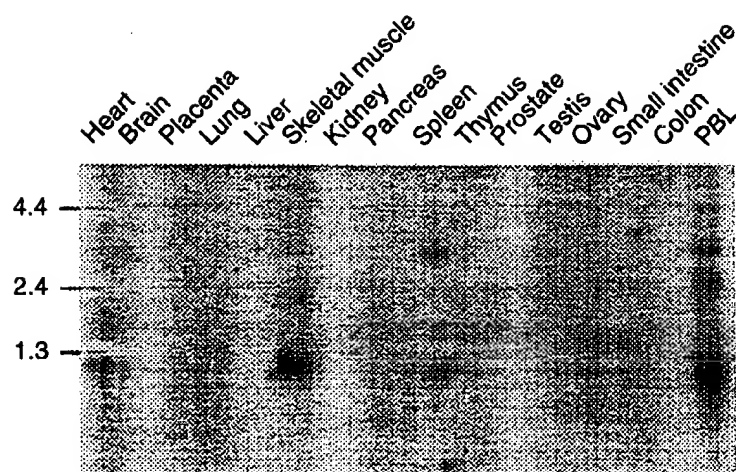
961 TGTTTGAAAGACTTCACTGTGGAAAAAATTCCTTTCCTTACCTNAAAAGGTNAAGGTA 1020
V *

1021 AGNNGCTGGCTNAGGNCNGGGCGCTGGANACTCTCTGNCCTGCNTCCCTCTGCTGTGTT 1080
1081 CCCACAGACAGAAANGCCTGNNCCTGNCCNAAGTCTGCTGTCTCNAGCCTGGCTCTATC 1140
1141 TTCTCCTTGTGATCGTCCCATCCCANATCCCGTGCACCCCCAGGACCTGGTCTCAT 1200
1201 CAGTCCCTCTCCTGGAGCTGGGGGTCCACACATCTCCAGCCAAGTCCAAGAGGCAGGGC 1260
1261 CAGTTCCTCCCATCTTCAGGCCCAGCCAGGCAGGGGGCAGTCGGCTCCTCAACTGGGTNA 1320
1321 CAAGGGTGAGGATGAGAAGTGGTCACGGGATTATTACGCCTT 1363



Northern Blot
TRAIL-R2

Fig. 3



Northern Blot
TRAIL-R3

Fig. 4

1	MAPPPARVHLGAF LAVTPNPGSAA SGTEAAAA TSPK VWS SAGRIE PRGGRGALPTSMGQH PSAR . ARAC PGPRPA
1 NEQRGON PAASGARKR PGPRPA
1
80	REASPRLRVHKTFKPVVAVVLLQVVPSSAAATIKLH... DOISIGTQQWEHSPLGELCPPGSHRSEPPGACNRCCTEGVGYTN
26	RGARPGRLVRPKTIVVAVVLLVWSAESALITQQA POORVAPQQRKRSPESEGLCPPGCHHISE DGRDCISCKYGGDYIT
1	.. MARIPTTLKFVIVAVVLEFAYSAITARQEE. POORVAPQQRHRHSFKGEECPAGSHRSEITGACNRCCTEGVGYTN
157	ASNNLFACLPCTACKSDEEEISPCCTTTRNTACQCKPGTFRNENSAEMCRKCKSTGCPRGMVKVKDCTPMSDIECVHKESGN
106	HWNZLLFCLRCRCDSGREVELSPCCTTTRNTVQCCEEGTFRREE SPENCRKCKRTGCPRGMVKVGDDCTPMSDIECVHKESCT
78	ASNNEPSCFPCVCKSDCKHSSCTMTTRTVQCCKEGTFRNENSPENCRKCSR . CPSEGEVQVSCTSMDDIOCVEEFQFN
237	GH
186	KHSGEAPAVEETITSPGTPASPCS.....
157	KT . VETPAAEETNNSPGTPAPAAEETMTTSPGTPAPAAEETMTTSPGTPAPAAEETMTTSPGTPAPAAEETMTTSPGTP
239 NIMTITVTVPVLIIVAVLVC..... CCI GSGCGGDPKCDRVCFWRLGLLRGPCEADNAHNEI SNAD
211	.. LSCITIGVTAAHVAVFVCKSLWKVLPYLKICSGGGDEFRNDRSS..... QRPGEADNVLNEI SILO
236	ASSHYLSCTHVGIVVLTIVFV.....
305	SLSTFV8EQQNESOEPAITLGTVTOSPGEAQCLLGPAAEAGCSQRRRLLV PANCDPTETLMLFFDKFANTVPFFDSWQLM
281	P..TCVPEQENEOEPAITGVNLSLPGSESEHLLPEAAERSQRRRLLV PANEDPTETLRQCFFDFADVPFFDSWFLM
385	RQLDLTKNEIDVVAGTACPGDALYAMLKWVNKTRGNASHTLLDALEREERHAKKEKIQDLLDSCKFFYLEGDTGCSA
359	RRLGLMDNEIKVANAFAAGHRETLITMLKWNKTRGRDASHHTLLDALETCERLAKOKIEDHLLSSCKFFYLEGNADSA
465	SLE
439	S...

TRAIL-R 2/3
Fig. 5

-1-

SEQUENCE LISTING

SEQ ID NO:1

	1	CGGAGAACCC	CGCAATCTCT	GCGCCACAA	AATACACCGA	CGATGCCCGA
	51	TCTACTTTAA	GGGCTGAAAC	CCACGGGCCT	GAGAGACTAT	AAGAGCGTTC
5	101	CCTACCGCCA	TGGAACAACG	GGGACAGAAC	GCCCCGGCCG	CTTCGGGGGC
	151	CCGAAAAGG	CACGGCCCAG	GACCCAGGGA	GGCGCGGGGA	GCCAGGCCTG
	201	GGCTCCGGGT	CCCCAAGACC	CTTGTGCTCG	TTGTGCGCCG	GGTCCTGCTG
	251	TTGGTCTCAG	CTGAGTCTGC	TCTGATCACC	CAACAAGACC	TAGCTCCCCA
	301	GCAGAGAGTG	GCCCCACAAC	AAAAGAGGTC	CAGCCCCTCA	GAGGGATTGT
10	351	GTCCACCTGG	ACACCATATC	TCAGAAGACG	GTAGAGATTG	CATCTCCTGC
	401	AAATATGGAC	AGGACTATAG	CACTCACTGG	AATGACCTCC	TTTTCTGCTT
	451	GCGCTGCACC	AGGTGTGATT	CAGGTGAAGT	GGAGCTAAGT	CCCTGCACCA
	501	CGACCAGAAA	CACAGTGTGT	CAGTGCGAAG	AAGGCACCTT	CCGGGAAGAA
	551	GATTCTCCTG	AGATGTGCCG	GAAGTGCCGC	ACAGGGTGTC	CCAGAGGGAT
15	601	GGTCAAGGTC	GGTGATTGTA	CACCCCTGGAG	TGACATCGAA	TGTGTCCACA
	651	AAGAATCAGG	TACAAAGCAC	AGTGGGGAAG	CCCAGCTGT	GGAGGACAGC
	701	GTGACCTCCA	GCCCAGGGAC	TCCTGCCTCT	CCCTGTTCTC	TCTCAGGCAT
	751	CATCATAGGA	GTCACAGTTG	CAGCCGTAGT	CTTGATTGTG	GCTGTGTTTG
	801	TTTGCAAGTC	TTTACTGTGG	AAGAAAGTCC	TTCTTTACCT	GAAAGGCATC
20	851	TGCTCAGGTG	GTTGGTGGGA	CCCTGAGCGT	GTGGACAGAA	GCCTCACAACG
	901	ACCTGGGGCT	GAGGACAATG	TCCTCAATGA	GATCGTGAGT	ATCTTGCAGC
	951	CCACCCAGGT	CCCTGAGCAG	GAAATGGAAG	TCCAGGAGCC	AGCAGAGCCA
	1001	ACAGGTGTCA	ACATGTGTGC	CCCCGGGGAG	TCAGAGCATC	TGCTGGAACC
	1051	GGCAGAAGCT	GAAAGGTCTC	AGAGGAGGAG	GCTGCTGGTT	CCAGCAAATG
25	1101	AAGGTGATCC	CACTGAGACT	CTGAGACAGT	GCTTCGATGA	CTTTGCAGAC
	1151	TTGGTGCCCT	TTGACTCCTG	GGAGCCGCTC	ATGAGGAAGT	TGGGCCCTCAT
	1201	GGACAATGAG	ATAAAGGTGG	CTAAAGCTGA	GGCAGCGGGC	CACAGGGACA
	1251	CCTTGTACAC	GATGCTGATA	AAGTGGGTCA	ACAAAACCGG	GCGAGATGCC
	1301	TCTGTCCACA	CCCTGTCTGA	TGCCTTGGAG	ACGCTGGGAG	AGAGACTTGC
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	1451	AGTCAGACCT	TCCCTGGTTT	ACCTTTTTTC	TGGAAAAAGC	CCAACCTGGAC
	1501	TCCAGTCAGT	AGGAAAGTGC	CACAATTGTC	ACATGACCGG	TACTGGAAGA
	1551	AACTCTCCCA	TCCAACATCA	CCCAGTGGAT	GGAACATCCT	GTAACTTTTT
35	1601	ACTGCACTTG	GCATTATTTT	TATAAGCTGA	ATGTGATAAT	AAGGACACTA
	1651	TGGAATGTG	TGGATCATTC	CGTTGTGCG	TACTTTGAGA	TTTGGTTTGG
	1701	GATGTCATTG	TTTTCACAGC	ACTTTTTTAT	CCTAATGTAA	ATGCTTTTAT
	1751	TATTTATTTG	GGCTACATTG	TAAGATCCAT	CTACACAGTC	GTTGTCCGAC
	1801	TTCACTTGAT	ACTATATGAT	ATGAACCTTT	TTTGGGTGGG	GGGTGCGGGG
40	1851	CAGTTCACTC	TGTCTCCAG	GCTGGAGTGC	AATGGTGCAA	TCCTGGCTCA
	1901	CTATAGCCTT	GACCTCTCAG	GCTCAAGCGA	TTCTCCCACC	TCAGCCATCC
	1951	AAATAGCTGG	GACCACAGGT	GTGCACCACC	ACGCCCCGGT	AATTTTTTGT
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	2051	TCCTGGACTC	AAGCAGTCTG	CCCACCTCAG	ACTCCCAAAG	CGGTGGAATT
45	2101	AGAGGCGTGA	GCCCCATGCT	TGGCCTTACC	TTTCTACTTT	TATAATTCTG
	2151	TATGTTATTA	TTTTATGAAC	ATGAAGAAAC	TTTAGTAAAT	GTACTTGTTT
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	2251	TGGGGGAAGA	AGAAGAAGTC	CCCTGTAAGA	TGTCACTGTC	TGGGTTCCAG
	2301	CCCTCCCTCA	GATGTACTTT	GGCTTCAATG	ATTGGCAACT	TCTACAGGGG
50	2351	CCAGTCTTTT	GAAGTGGACA	ACCTTACAAG	TATATGAGTA	TTATTTATAG
	2401	GTAGTTGTTT	ACATATGAGT	CGGGACCAAA	GAGAACTGGA	TCCACGTGAA
	2451	GTCCTGTGTG	TGGCTGGTCC	CTACCTGGGC	AGTCTCATTT	GCACCCATAG
	2501	CCCCCATCTA	TGGACAGGCT	GGGACAGAGG	CAGATGGGTT	AGATCACACA
	2551	TAACAATAGG	GTCTATGTCA	TATCCCAAGT	GAAGTTGAGC	CCTGTTTGGG
55	2601	CTCAGGAGAT	AGAAGACAAA	ATCTGTCTCC	CACGTCTGCC	ATGGCATCAA
	2651	GGGGGAAGAG	TAGATGGTGC	TTGAGAATGG	TGTGAAATGG	TTGCCATCTC
	2701	AGGAGTAGAT	GGCCCCGGCT	ACTTCTGGTT	ATCTGTCACC	CTGAGCCCCAT

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5 2751 GAGCTGCCTT TTAGGGTACA GATTGCCTAC TTGAGGACCT TGGCCGCTCT
 2801 GTAAGCATCT GACTCATCTC AGAAATGTCA ATTCTTAAAC ACTGTGGCAA
 2851 CAGGACCTAG AATGGCTGAC GCATTAAGGT TTTCTTCTTG TGTCCTGTTC
 2901 TATTATTGTT TTAAGACCTC AGTAACCATT TCAGCCTCTT TCCAGCAAAC
 2951 CCTTCTCCAT AGTATWTCAG TCATGGAAGG RTCATTTATG CAGGTAGTCA
 3001 TTCCAGGAGT TTTTGGTCTT TTCTGTCTCA AGGCATTGTG TGTTTTGTTC
 3051 CGGGACTGGT TTGGGTGGGA CAAAGTTAGA ATTGCCTGAA GATCACACAT
 10 3101 TCAGACTGTT GTGTCTGTGG AGTTT TAGGA GTGGGGGGTG ACCTTTCTGG
 3151 TCTTTGCACT TCCATCCTCT CCCACTTCCA TCTGGCATCC CACGCGTTGT
 3201 CCCCTGCACT TCTGGAAGGC ACAGGTGCT GCTGCCCTCT GGTCTTTGCC
 3251 TTTGCTGGGC CTTCTGTGCA GGACGCTCAG CCTCAGGGCT CAGAAGGTGC
 3301 CAGTCCGGTC CCAGGTCCCT TGTCCTTCC ACAGAGGCTC TCCTAGAAGA
 3351 TGCATCTAGA GTGTCAGCCT TATCAGTGT TAAGATTTGT CTTTTATTTT
 3401 TAATTTTTTT GAGACAGAAT CTCACTCTCT CGCCCAGGCT GGAGTGCAAC
 15 3451 GGTACGATCT TGGCTCAGT CAACCTCCGC CTCTGGGTT CAAGCGATTC
 3501 TCGTGCCTCA GCCTCCGG

SEQ ID NO:2

20 1 MEQRGQNA PA ASGARKRHGP GPREARGARP GLRVPKTLVL VVAAVLLLVLS
 51 AESALITQOD LAPQQRVAPQ QKRSSPSEGL CPPGHHISED GRDCISCKYG
 101 QDYSTHWNDL LFCLRCTRCD SGEVELSPCT TTRNTVCQCE EGTFREEDSP
 151 EMCRKCRGTG PRGMVKVGDG TPWSDIECVH KESGTHKSGE APAVEETVTS
 201 SPGTPASPCS LSGIIIGVTV AAVVLIVAVF VCKSLLWKV LPYLKIGCSG
 251 GGGDPERVDR SSQRPGAEDN VLNEIVSILQ PTQVPEQEME VOEPAEPTGV
 301 NMLSPGESEH LLEPAEAERS QRRRLVLPAN EGDPTETLRQ CFDDFADLVP
 25 351 FDSWEPLMRK LGLMDNEIKV AKAEAAAGHRD TLYTMLIKWV NKTGRDASVH
 401 TLLDALETG ERLAKQKIED HLLSSGKFMV LEGNADSAMS

SEQ ID NO:3

30 1 TTCGGCACGA GGCACGCGCA CGAACTCAGC CAACGATTTC TGATAGATTT
 51 TTGGGAGTTT GACCAGAGAT GCAAGGGGTG AAGGAGCGCT TCCTACCGTT
 101 AGGAACTCTG GGGACAGAGC GCGGCGGCG CCTGATGGCC GAGGCAGGGT
 151 GCGACCCAGG ACCCAGGACG GCGTCGGGAA CCATACCATG GCGCGGATCC
 201 CCAAGACCCT AAAGTTCGTC GTCGTATCG TCGCGGTCCT GCTGCCAGTC
 251 CTAGCTTACT CTGCCACCAC TGCCCGGCAG GAGGAAGTTC CCCAGCAGC
 301 AGTGGCCCA CAGCAACAGA GGCACAGCTT CAAGGGGGAG GAGGTGCCAG
 35 351 CAGGATCTCA TAGATCAGAA CATACTGGAG CCTGTAACCC GTGCACAGAG
 401 GGTGTGGATT ACACCAACGC TTCCAACAAT GAACCTTCTT GCTTCCCATG
 451 TACAGTTTGT AAATCAGATC AAAAACATAA AAGTTCCTGC ACCATGACCA
 501 GAGACACAGT GTGTCACTGT AAAGAAGGCA CCTTCCGGAA TGTTAACTCC
 551 CCAGAGATGT GCCGGAAGTG TAGCAGGTGC CCTAGTGGGG AAGTCCAAGT
 40 601 CAGTAATTGT ACGTCCTGGG ATGATATCCA GTGTGTTGAA GAATTTGGTG
 651 CCAATGCCAC TGTGGAAACC CCAGCTGCTG AAGAGACAAT GAACACCAGC
 701 CCGGGGACTC CTGCCCCAGC TGCTGAAGAG ACAATGAACA CCAGCCCAGG
 751 GACTCCTGCC CCAGCTGCTG AAGAGACAAT GACCACCAGC CCGGGGACTC
 801 CTGCCCCAGC TGCTGAAGAG ACAATGACCA CCAGCCCAGG GACTCCTGCC
 45 851 CCAGCTGCTG AAGAGACAAT GACCACCAGC CCGGGGACTC CTGCCTCTTC
 901 TCATTACCTC TCATGCACCA TCGTAGGGAT CATAGTTCTA ATTGTGCTTC
 951 TGATTGTGTT TGTTTGAAAG ACTTCACTGT GGAAGAAATT CCTTCCTTAC
 1001 CTGAAAGGTT CAGGTAGGCG CTGGCTGAGG GCGGGGGGCG CTGGCACTC
 1051 TCTGCCCTGC CTCCCTCTGC TGTGTTCCCA CAGACAGAAA CGCCTGCCCC
 50 1101 TGCCCCAAGT CCTGGTGTCT CCAGCCTGGC TCTATCTTCC TCCTTGTGAT
 1151 CGTCCCATCC CCACATCCCG TGCACCCCCC AGGACCCTGG TCTCATCAGT
 1201 CCCTCTCCTG GAGCTGGGGG TCCACACATC TCCAGCCAA GTCCAAGAGG
 1251 GCAGGGCCAG TTCCTCCCAT CTTCAGGCCC AGCCAGGCCA GGGGGCAGTC
 1301 GGCTCCTCAA CTGGGTGACA AGGGTGAGGA TGAGAAGTGG TCACGGGATT
 55 1351 TATTCAGCCT T

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SEQ ID NO:4

5
1 MARIPKTLKF VVIVAVLLP VLAYSATTAR QEEVPQQTVA PQQQRHSFKG
51 EEC PAGSHRS EHTGACNPCT EGVDTNASN NEPSCFPCTV CKSDQKHKSS
101 CTMTRDTCVQ CKEGTFRNVN SPEMCRKCSR CPSGEVQVSN CTSWDDIQC
151 EEFGANATVE TPAAEETMNT SPGTPAPAAE ETMNTSPGTP APAAEETMTT
201 SPGTPAPAAE ETMTTSPGTP APAAEETMTT SPGTPASSHY LSCTIVGIIV
251 LIVLLIVFV

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